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Full Length Research Paper

Appraisal of the antioxidant, phenolic compounds concentration, xanthine oxidase and tyrosinase inhibitory activities of *Pleurotus salmoneostramineus*

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The purpose of this study was to evaluate the antioxidant activities, tyrosinase inhibitory effects on the fruiting bodies of *Pleurotus salmoneostramineus* extracted with acetone, methanol and hot water. The antioxidant activities were performed on -carotene-linoleic acid, reducing power, DPPH, ferrous ions chelating abilities, and xanthine oxidase inhibitory activities. In addition to this, phenolic compounds were also detected. Acetonic, methanolic and hot water extracts of *P. salmoneostramineus* showed the similar pattern of -carotene- linoleic acid inhibition. At 8 mg/ml, methanolic extract showed a high reducing power of 1.62. The scavenging effects on DPPH, acetonic and methanolic extracts were effective than hot water extract. The strongest chelating effect was obtained from the acetonic and methanolic extracts at 1.0 mg/ml. Gallic acid, protocatechuic acid, chlorogenic acid, formononetin, and biochanin-A were detected from acetonitrile and 0.1N hydrochloric acid (5:1) solvent extract. The xanthine oxidase and tyrosinase inhibitory activities of the acetonic, methanolic, and hot water extracts increased with increasing concentration. The results suggested that fruiting bodies of *P. salmoneostramineus* may have potential as a natural antioxidants.

Key words: Antioxidant, phenolic compounds, *Pleurotus salmoneostramineus*, tyrosinase inhibition, xanthine oxidase.

INTRODUCTION

Pleurotus salmoneostramineus, commonly known as pink oyster mushroom, grows on dead deciduous plants (Murakami and Takemaru, 1990). It is becoming popular due to attractive color, sustainable yield, delicious test, and unique texture. *P. salmoneostramineus* is rich in protein, lipids, fiber, carbohydrates, vitamins, and contains an abundant amount of essential amino acids (Shibata et al., 1997; Bao et al., 2004). Oxidative stress depicts the existence of products called free radicals and reactive oxygen species (ROS), which are formed under normal physiological conditions, but become deleterious when not being eliminated by the endogenous systems. In fact, oxidative stress results from an imbalance between the generation of ROS and endogenous antioxidant systems.

ROS are major sources of primary catalysts that initiate oxidation *in vivo* and *in vitro* and create oxidative stress which results in numerous diseases and disorders (Rackova et al., 2007). Oxygen derived free radicals such as superoxide anions, hydroxyl radicals and hydrogen peroxide are cytotoxic and give rise to tissue injuries (Jainu and Shyamala, 2005). Excessive amount of ROS is harmful because they initiate bimolecular oxidation which leads to cell death and creates oxidative stress. In addition, oxidative stress causes inadvertent enzyme activation and oxidative damage to cellular system (Wiseman and Halliwell, 1996). Xanthine oxidase inhibitors are much useful, since they possess lesser side effects compared to uricosuric and anti-inflammatory agents. A potential source of such compounds can be obtained from mushroom. Flavonoids and polyphenolic crude extracts

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Abbreviations: BHT, butylated hydroxytoluene; **DMSO,** dimethyl sulfoxide; **DPPH,** 1,1-diphenyl-2-picrylhydrazyl; **L-DOPA,** L-phenylalanine (L-DOPA); **XO,** Xanthine oxidase.

Have been reported to possess xanthine oxidase inhibitory activity (Lio et al., 1985; Chang et al., 1993).

Tyrosinase is widely distributed in mushrooms, plants, and animal. It is responsible for melanization. Nonenzymatic oxidation can be protected by antioxidating additives and enzymatic oxidation can be prevented by tyrosinase inhibitors (Masuda et al., 2005). In spite of the medicinal importance of *P. salmoneostramineus* or the therapeutic potential, there have not been many studies on physiologically beneficial components. However, comprehensive studies on the antioxidant properties of this mushroom are not available. Therefore, the purpose of the present study is to evaluate the antioxidant potentials and tyrosinase inhibitory effects of acetonic, methanolic, and hot water extracts from the fruiting bodies of *P. salmoneostramineus*. Antioxidant activities were assayed including -carotene-linoleic acid, reducing power, scavenging effects on DPPH radicals, chelating effects on ferrous ions, and xanthine oxidase. The contents of phenolic acid and flavonoid components were also analyzed.

MATERIALS AND METHODS

Chemicals and reagents

-carotene, linoleic acid, chloroform, polyoxyethylene sorbitan monopalmitate (Tween40), butylated hydroxytoluene (BHT), -tocopherol (TOC), 1,1-diphenyl-2-picrylhydrazyl (DPPH), L-ascorbic acid, potassium ferricyanide, trichloroacetic acid, ferrous chloride, ferric chloride, ferrozine, Folin-Ciocalteu reagent, gallic acid, methanol, 3,4-dihydroxy-L-phenylalanine (L-DOPA), xanthine, allopurinol, mushroom tyrosinase, and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All chemicals and solvents were used as HPLC or analytical grade.

Mushroom and extraction

Fresh and mature fruiting bodies of *P. salmoneostramineus* were obtained from Mushroom Research Institute of Gyeonggi Province in Korea. A pure culture was deposited in Culture Collection and DNA Bank of Mushroom (CCDBM), Division of Life Sciences, University of Incheon, Korea and acquired accession number, IUM-3975. Fruiting bodies were dried with hot air at 40°C for 48 h and finely pulverized. 5 g of powdered samples were extracted with 100 ml of 60% acetone and 80% methanol with stirring at 150 rpm for 24 h at 25°C to obtain acetonic and methanolic extracts. The mixture was filtered through two layer of Whatman no. 1 filter paper. The same quantity of sample was boiled at 100°C for 3 h with 100 ml deionized distilled water to obtain a hot water extract. The mixture was cooled to room temperature and filtered through Whatman no. 1 filter paper. The residues were then extracted with two additional 100 ml aliquots of acetone, methanol, and deionized water, as described above.

The combined extracts were evaporated with a rotary evaporator (Eyela, Saitama, Japan) at 40°C, and the remaining solvent was removed with a freeze-drier (Optizen, Daejeon, Korea). The yields from the acetonic, methanolic and hot water extracts of *P. salmoneostramineus* were 22.64, 22.46, and 19.96% (w/w), respectively.

Antioxidant activity by -carotene-linoleic acid

Antioxidant activity was determined by measuring the inhibition of volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius et al., 1998) . A stock solution of a -carotene-linoleic acid mixture was prepared as follows 0.5 mg -carotene was dissolved in 1 ml of chloroform, and 25 l of linoleic acid and 200 mg Tween 40 was added. The chloroform was removed completely using a vacuum evaporator. Then, 100 ml of oxygenated distilled water was added with vigorous shaking; 2.5 ml of this reaction mixture was dispensed to test tubes, 0.5 ml of various concentrations (0.5 to 20.0 mg/ml) of the extracts in methanol was added, and the reaction mixture was incubated for up to 2 h at 50°C. The same procedure was repeated with the positive controls BHT and TOC, and a blank. After the incubation, the absorbance of the mixtures was measured at 490 nm using a spectrophotometer (Optizen POP; Mecasys Co. Ltd., Daejeon, Korea). The absorbance was measured until the -carotene color disappeared.

The -carotene bleaching rate (R) was calculated according to Equation (1):

$$R = \ln (a/b)/ t \quad (1)$$

Where, ln = natural log, a = absorbance at time t (0), b = absorbance at time t (120 min). The antioxidant activity (AA) was calculated as the percent inhibition relative to the control using Equation (2):

$$AA = [(R_{control} - R_{sample}) /R_{control}] \times 100 \quad (2)$$

Antioxidant activities of the extracts were compared with those of BHT and TOC at 0.5 mg/ml and a blank consisting of 0.5 ml methanol.

Reducing power

Reducing power was determined according to the method of Gulcin et al. (2003). Each extract (1 to 8 mg/ml) in methanol (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricynide, and the mixture was incubated at 50°C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid was added, and the mixture was centrifuged at 200 × g (6K 15; Sigma, Mannchein, Germany) for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. Finally, the absorbance was measured at 700 nm against a blank. BHT and TOC were used as positive controls.

Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl radicals

The hydrogen atoms or electron donation ability of the corresponding extracts and some pure compounds were measured from the bleaching of the purple colored DPPH methanol solution (Cuendet et al., 1997). 4 ml of various concentrations (0.125 to 2.0 mg/ml) of the extracts in methanol was added to 1 ml of DPPH radical solution in methanol (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and allowed to stand for 30 min, and the absorbance of the resulting solution was measured at 517 nm using a spectrophotometer. Inhibition of the DPPH free

radical in percent (I %) was calculated as:

$I\ \% = [(A_{control} - A_{sample})/ A_{control}] \times 100$

Where, $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. BHT, TOC, and L-ascorbic acid were used as positive controls.

Chelating effects on ferrous ions

The chelating effect was determined according to the method of Dinis et al. (1994). Briefly, 2 ml of various concentrations (0.063 to 1.0 mg/ml) of the extracts in methanol was added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by adding 5 mM ferrozine (0.2 ml). The total volume was adjusted to 5 ml with methanol, and the mixture was shaken vigorously and left at room temperature for 10 min. The absorbance of the solution was measured spectrophotometrically at 562 nm. The inhibition percentage of the ferrozine-Fe^{2+} complex formation was calculated using the following formula:

Metal chelating effect (%) = $[(A_{control} - A_{sample})/ A_{control}] \times 100$

Where, $A_{control}$ is the absorbance of the control (control contained $FeCl_2$ and ferrozine; complex formation molecules), and A_{sample} is the absorbance of the test compound. BHT and TOC were used as positive controls.

Analysis of phenolic compounds

Fifteen standard phenolic compounds, including gallic acid, pyrogallol, homogentisic acid, protocatechuic acid, (+) catechin, chlorogenic acid, caffeic acid, vanillin, ferulic acid, naringin, resveratrol, naringenin, hesperetin, formononetin, and biochanin-A were purchased from Sigma Aldrich and used for calibration curves. The standard stock solutions (50, 100, 250, and 500 ppm) were prepared in DMSO. Sample compounds were identified based on retention times of authentic standards and were quantified by comparing their peak areas with those of the standard curves. Sample preparation for the phenolic compound analysis followed Kim et al. (2006). 2 g of dried mushroom powder were mixed with 10 ml of acetonitrile and 2 ml of 0.1 N hydrochloric acid and stirred 150 rpm for 2 h at room temperature. The suspension was filtered through Whatman no. 42 filter paper. The extract was freeze-dried, and the residues were redissolved in 10 ml of 80% aqueous methanol (HPLC grade) and filtered through a 0.45 m nylon membrane filter (Titan, Rockwood, TN, USA). The 20 l filtrate was loaded onto an Agilent-1100 series liquid chromatography HPLC system (Agilent Technologies, Waldbronn, Germany).

Separation was achieved on a 250 nm × 4.6 mm i.d., 5 m, YMC-Pack ODS AM (YMC Co. Ltd., Kyoto, Japan) column. The mobile phase was distilled water with 0.1% glacial acetic acid (solvent A) and acetonitrile with 0.1% glacial acetic acid (solvent B). The gradient was 0 min, 92% A; 0 to 2 min, 90% A; 2 to 27 min, 70% A; 27 to 50 min, 10% A; 50 to 51 min, 0% A; 51 to 60 min, 0% A; 60 to 63 min, 92% A. The run time was 60 min using a flow rate of 1 ml/min. Detection was performed with a diode array detector at a wavelength of 280 nm.

Xanthine oxidase inhibition

In vitro xanthine oxidase (XO) inhibitory activity of various extracts from the fruiting bodies of *P. salmoneostramineus* was assayed spectrophotometrically under aerobic conditions using xanthine as the substrate (Owen and Johns, 1999). The assay mixture consisted of 1 ml extract of the different concentrations (0.5 to 8.0 mg/ml), 2.9 ml of phosphate buffer (pH 7.5), and 0.1 ml of xanthine oxidase enzyme solution (0.1 units/ml in phosphate buffer, pH 7.5), which was prepared immediately before use. After pre incubation at 25°C for 15 min, the reaction was initiated by the addition of 2 ml of the substrate solution (150 M xanthine in the same buffer). The assay mixture was incubated at 25°C for 30 min. The reaction was then stopped by the addition of 1 ml of 1N hydrochloric acid and the absorbance was measured at 290 nm using a spectrophotometer. Different concentrations of the extracts were dissolved in DMSO and the final concentration of DMSO was 5%, which did not affect the enzyme assay.

Proper controls with DMSO were carried out. Allopurinol (0.5 to 8.0 mg/ml), a known inhibitor of XO, was used as positive control. One unit of XO is defined as the amount of enzyme required to produce 1 mmol of uric acid/min at 25°C. Xanthine oxidase inhibitory activity was expressed as the percentage inhibition of XO in the above assay system calculated as:

Inhibition (%) = $[(A - B) - (C - D)/ (A - B)] \times 100$

Where A is the activity of the enzyme without the extraction, B is the control of A without the extraction and enzyme; C and D are the activities of the extraction with and without XO, respectively.

Tyrosinase inhibition

Tyrosinase inhibition activity was determined using the modified dopachrome method with L-DOPA as the substrate (Masuda et al., 2005). A 96-well microtiter plate was used to measure absorbance at 475 nm with 700 nm as a reference. Extract fractions were dissolved in 50% DMSO. Each well contained 40 l of sample with 80 l of phosphate buffer (0.1 M, pH 6.8), 40 l of tyrosinase (31 units/ml), and 40 l of L-DOPA (2.5 mM). The mixture was incubated for 10 min at 37°C, and absorbance was measured at 475 nm using a UVM 340 microplate reader (Asys, Eugendrof, Austria). Each sample was accompanied by a blank containing all components except L-DOPA. L-ascorbic acid and kojic acid were used as positive controls. The results were compared with a control consisting of 50% DMSO in place of the sample. The percentage of tyrosinase inhibition was calculated as follows:

$[(A_{control} - A_{sample})/ A_{control}] \times 100\%$

Statistical analysis

Data were expressed as means ± standard deviations of three replicate determinations and were analyzed by SPSS V.13 (SPSS Inc., Chicago, IL, USA). A one way analysis of variance and Duncan's new multiple-range test were used to determine the differences among the means.

RESULTS AND DISCUSSION

Antioxidant activity on -carotene-linoleic acid

Polyunsaturated fatty acids, such as linoleic acid, are easily oxidized by the oxygen in the air. This auto-oxidation leads to the occurrence of chain reactions with

Table 1. Antioxidant activity against -carotene-linoleic acid of different concentrations of various extracts from the fruiting bodies of *Pleurotus salmoneostramineus.*

Solvent and control	Sample concentration (mg/ml)			
	0.5	2.0	8.0	20.0
Acetone	68.73 ± 0.62	77.35 ± 0.07	90.19 ± 0.06	95.45 ± 0.05
Methanol	63.82 ± 0.19	78.39 ± 0.16	91.90 ± 0.05	95.17 ± 0.04
Hot water	52.97 ± 0.14	75.54 ± 0.16	89.39 ± 0.08	94.37 ± 0.03
BHT	95.21 ± 0.17	-	-	-
TOC	96.02 ± 0.18	-	-	-

Values expressed as means ± SD (n = 3). -, not analyzed; BHT, butylated hydroxytoluene; TOC, -tocopherol.

Table 2. Reducing power of different concentrations of various extracts from the fruiting bodies of *Pleurotus salmoneostramineus*.

Solvent and control	Sample concentration (mg/ml)			
	1.0	2.0	4.0	8.0
Acetone	0.376 ± 0.02	0.596 ± 0.03	0.898 ± 0.17	1.329 ± 0.19
Methanol	0.539 ± 0.07	0.794 ± 0.18	1.202 ± 0.23	1.619 ± 0.22
Hot water	0.343 ± 0.02	0.483 ± 0.11	0.718 ± 0.17	1.231 ± 0.21
BHT	3.212 ± 0.49	-	-	-
TOC	2.162 ± 0.32	-	-	-

Values expressed as means ± SD (n = 3). -, not analyzed; BHT, butylated hydroxytoluene; TOC, -tocopherol.

the formation of coupled double bonds, and at a later stage also obtaining secondary products, such as aldehydes, ketones, and alcohols. Using the -carotene-linoleic acid method, the acetonic, methanolic and hot water extracts of *P. salmoneostramineus* showed similar patterns of antioxidant activities. Acetonic extract showed the strongest linoleic acid inhibition capacity (95.45%) at 20.0 mg/ml (Table 1). The antioxidative components in the mushroom extracts can reduce the extent of -carotene destruction by neutralizing the linoleate free radical and other free radicals formed in the system.

Barros et al. (2007) reported that the antioxidant activities of *Leucopaxillus giganteus*, *Sarcodon imbricatus* and *Agaricus arvensis* in various extracts increased with increasing concentration. Their antioxidant activities were 61.4, 54.3 and 46.7% at 5 mg/ml, while antioxidant activity of TBHQ (tertiary butylhydroquinone) standard reached 82.2% at 2 mg/ml. It seems that the antioxidant activity of *P. salmoneostramineus* fruiting bodies was more effective than those mentioned above.

Reducing power

The reducing power of *P. salmoneostramineus* in acetonic, methanolic, and hot water extracts and as a function of their concentration is shown in Table 2. The reducing power was increased with increasing concentration. At mg/ml, the strongest reducing power inhibition was determined in methalonic extract a value of 1.62 and the lowest reducing power inhibition (1.23) was exhibited by the hot water extract. Reducing power of synthetic antioxidant, BHT and TOC at 1.0 mg/ml were 3.21 and 2.16, respectively (Table 2). Lee et al. (2007) reported that edible mushrooms, *Hypsizygus marmoreus*, *Agricus bisporus*, *Pleurotus eryngii*, *Pleurotus ferulae* and *Pleurotus ostreatus* showed reducing powers of 0.99, 0.76, 0.75, 0.70 and 0.61 at 20 mg/ml, respectively. It can be seen that the reducing power of *P. salmoneostramineus* was higher than those of *H. marmoreus*, *A. bisporus*, *P. eryngii*, *P. ferulae* and *P. ostreatus*.

The reducing power properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Shimada et al., 1992; Barros et al., 2007).

Scavenging effect on DPPH

Scavenging effects of the acetonic, methanolic, and hot water extracts from the fruiting bodies of *P. salmoneostramineus* on DPPH radicals increased with increasing concentration. At 0.125 to 2.0 mg/ml, the scavenging activities of the acetonic, methanolic, and hot

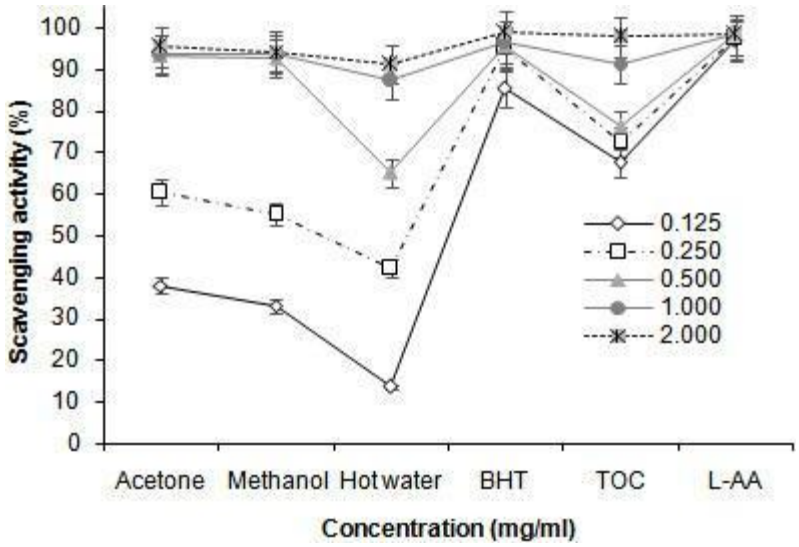

Figure 1. Scavenging activity of various extracts from the fruiting bodies of *Pleurotus salmoneostramineus* against 1,1-diphenyl-2-picrylhydrazyl. Values expressed as means ± SE (n = 3). BHT, butylated hydroxytoluene; TOC, -tocopherol; L-AA, L-ascorbic acid.

water extracts of *P. salmoneostramineus* on DPPH radical ranged from 37.99 to 95.36%, 33.18 to 94.26%, and 13.53 to 91.07%, respectively (Figure 1). Results indicate that acetonic, methanolic, and hot water extracts, respectively showed good, moderate, and poor activities at the concentration tested. However, at 0.125 to 2.0 mg/ml, BHT, TOC, and L-ascorbic acid showed the excellent scavenging activities of 85.25 to 98.74%, 67.37 to 97.78%, and 96.74 to 98.23%, respectively. With regard to ethanolic extracts of *H. marmoreus*, *A. bisporus* and *Pleurotus citrinopileatus* fruiting bodies scavenged DPPH radicals by 46.6 to 68.4% at 5 mg/ml (Lee et al., 2007). For cold and hot water extracts, at 20 mg/ml, the scavenging activities of fruiting bodies, mycelia and filtrate were 20.7 to 52.3%, 37.6 to 48.3%, and 19.6 to 23.3%, respectively.

It seems that the scavenging activity of *P. salmoneostramineus* fruiting bodies was more effective than those mentioned above. Various extracts might react with free radicals, particularly the peroxy radicals, which are the major propagators of the autoxidation chain of fat, thereby terminating the chain reaction (Frankel, 1991; Shahidi and Wanasundara, 1992). Antioxidant activity of natural antioxidants has been shown to be involved in termination of free radical reaction (Shimada et al., 1992). Furthermore, Herraiz et al. (2003) found that an essential amino acid L-tryptophan could react with phenolic aldehydes in food to form phenolic tetrahydro- - carboline alkaloids that scavenged 2,2-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid effectively. Therefore, the presence of L-tryptophan in various extracts might most likely account for the scavenging activity on DPPH radicals. However, the better activity of acetone extract might be due to more hydrogen-donating components contained within the extracts.

Chelating effects on ferrous ions

In the present study, the chelating activity of the acetonic, methanolic, and hot water extracts at five different concentrations (0.063, 0.125, 0.250, 0.500, and 1.000 mg/ml) from the fruiting bodies of *P. salmoneostramineus* toward ferrous ions was investigated. BHT and TOC were used as reference standards on ferrous ions. As can be seen from the Figure 2, chelating capacity of the extracts increased with increasing concentration. The strongest chelating effect (87.80 and 87.72%) obtained from the methanolic and acetonic extracts, respectively at 1.0 mg/ml. At this concentration, the lowest chelating effect was exhibited by hot water extract (78.02%). All of the extracts evaluated here showed significantly higher chelating effects on ferrous ions than those of the standards, BHT and TOC at the lower concentrations. With regard to hot water extracts at 20 mg/ml, *Ganoderma tsugae* and *Agrocybe cylindracea* chelated ferrous ions by 39.5 to 42.6% and 45.8%, respectively (Mau et al., 2005; Tsai et al., 2006). At 1 to 5 mg/ml, chelating abilities of *H . marmoreus* and *P*. *citrinopileatus* were 75.6-92.6% (Lee et al., 2007).

It seems that chelating ability of *P. salmoneostramineus* on ferrous ions was similar to that of *H. marmoreus* and *P. citrinopileatus*, while more effective than those of *G. tsugae* and *A. cylindracea*. Chelating

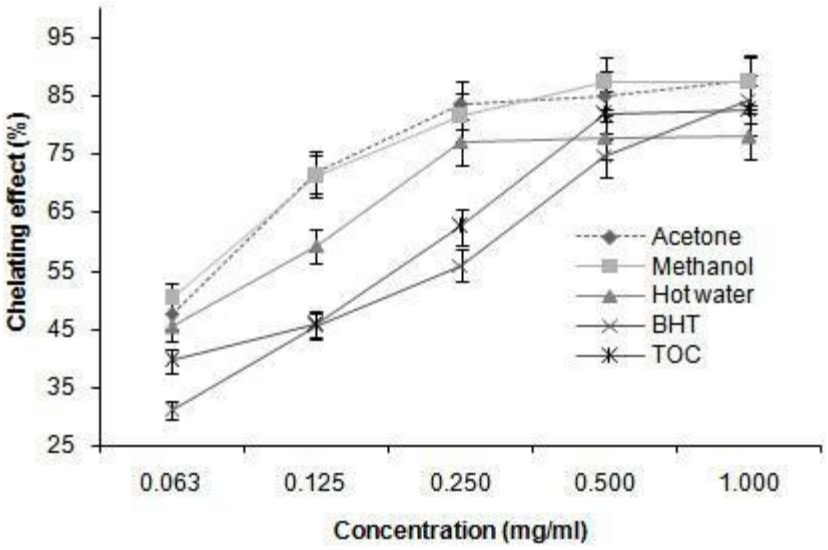

Figure 2. Chelating effect of various extracts from the fruiting bodies of *Pleurotus salmoneostramineus*. Values expressed as means ± SE (n = 3). BHT, butylated hydroxytoluene; TOC, -tocopherol.

agents may serve as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ions. Since ferrous ions were the most effective pro-oxidants in food system, (Yamaguchi et al., 1988), the high ferrous-ion chelating abilities of the various extracts from the fruiting bodies of *P. salmoneostramineus* would be beneficial.

Analysis of phenolic compound

Gallic acid, pyrogallol, homogentisic acid, protocatechuic acid, (+) catechin, chlorogenic acid, caffeic acid, vanillin, ferulic acid, naringin, resveratrol, naringenin, hesperetin, formononetin, and biochanin-A were used as standard for the detection of phenolic compounds from the extract of *P. salmoneostramineus*. Five phenolic compounds, gallic acid, protocatechuic acid, chlorogenic acid, formononetin, and biochanin-A were detected from the extract (Figure 3). The highest concentration of phenolic compound was recorded in protocatechuic acid (38 g/g) and followed by gallic acid (29 g/g), chlorogenic acid (28 g/g), biochanin-A (10 g/g), and formononetin (8 g/g). These findings are comparable to the previous studies on *P. ostreatus* (Kim et al., 2008) in which, six phenolic compounds such as, gallic acid, homogentisic acid, protocatechuic acid, chlorogenic acid, naringin, and myricetin were detected.

Mushroom species also contained different types of phenolic compound in varying numbers ranging from 3 to 15, while gallic acid was reported common phenolic compound in mushrooms. Thus, the content of phenolic compounds could be used as an important indicator of antioxidant capacity. Several reports have convincingly shown a close relationship between antioxidant activity and phenolic content (Duan et al., 2007; Pan et al., 2008; Zhao et al., 2006). Mushroom extracts have high levels of phenolic compounds, which are composed of one or more aromatic rings which bearing one or more hydroxyl groups, which can exhibit extensive free radical-scavenging activities as hydrogen donors or electron-donating agents, and metal ion-chelating properties. The greater numbers of hydroxyl groups in the phenolics could exhibit higher antioxidant activity (Prasad et al., 2005; Rangkadilok et al., 2007).

Xanthine oxidase inhibitory activity

Xanthine oxidase inhibitory activities of various extracts of *P. salmoneostramineus* increased with increasing concentration. At 0.5 to 8.0 mg/ml, the xanthine oxidase inhibition of the acetonic, methanolic, and hot water extracts ranged from 5.06 to 56.25%, 3.96 to 53.04%, and 4.01 to 52.26%, respectively. However, at the same concentrations, allopurinol showed the excellent xanthine oxidase inhibitory activity of 92.31 to 94.58% (Figure 4). Results indicate that acetonic extract showed good, while hot water and methanolic extracts showed moderate activities at the concentration tested. However, at higher doses of the extraction, xanthine oxidase would be significantly inhibited. Flavonoids are a group of polyphenolic compounds, which have been reported to possess xanthine oxidase inhibitory activity (Costantino et al., 1992). Hence, the presence of phenolic and flavonoid content in the extract would have contributed

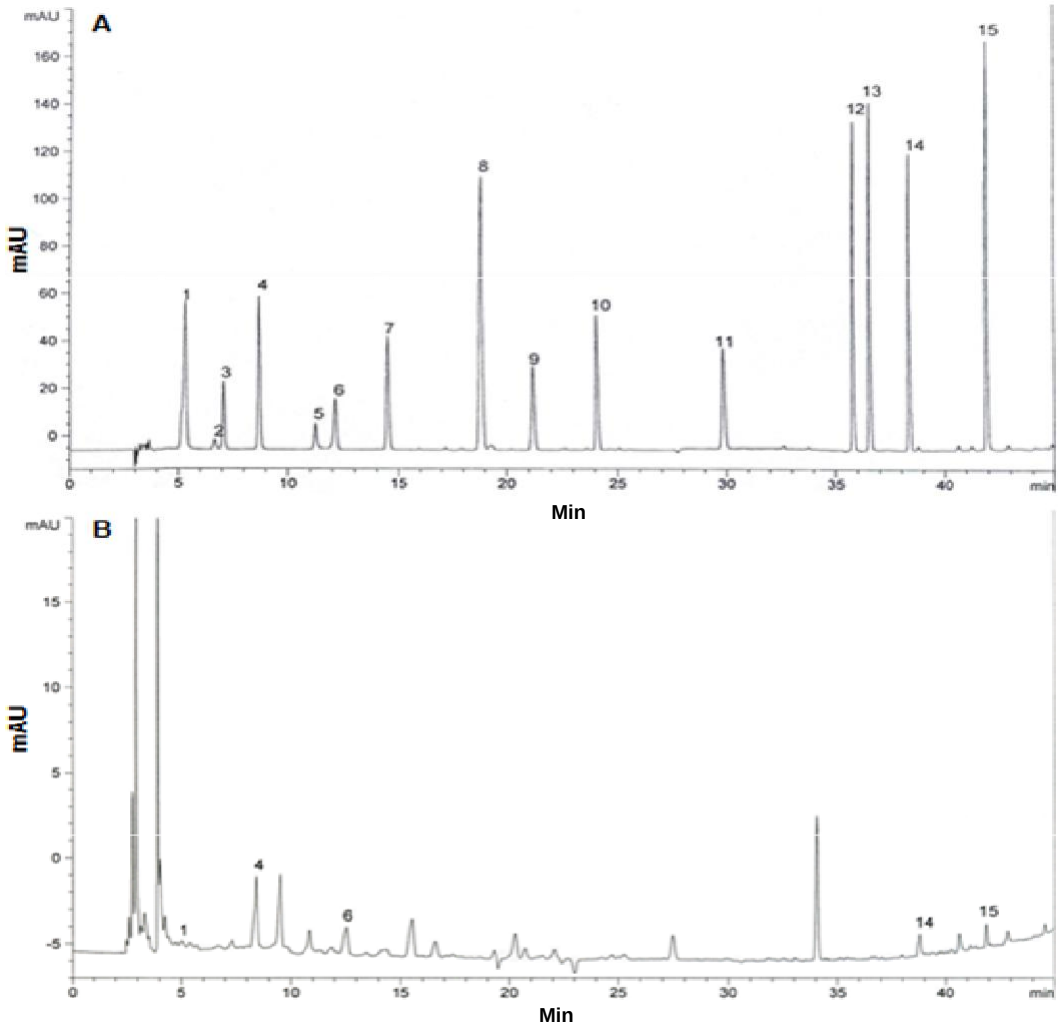

Figure 3. High performance liquid chromatography of phenolic compounds. A, Standard mixture of 15 phenolic compounds; B, *Pleurotus salmoneostramineus* extract. 1, gallic acid; 2, pyrogallol; 3, homogentisic acid; 4, protocatechuic acid; 5, (+) catechin; 6, chlorogenic acid; 7, caffeic acid; 8, vanillin; 9, ferulic acid; 10, naringin; 11, resveratrol; 12, naringenin; 13, hesperetin; 14, formononetin; 15, biochanin-A.

towards xanthine oxidase inhibition.

Tyrosinase inhibition

Tyrosinase inhibitory activities of the acetonic, methanolic, and hot water extracts from the fruiting bodies of *P. salmoneostramineus* increased with increasing concentration. At 0.125 to 1.0 mg/ml, the tyrosinase inhibition of acetonic, methanolic, and hot water extracts ranged from 19.30 to 58.78%, 20.90 to 63.29%, and 15.25 to 49.25%, respectively (Figure 5). Results indicate that methanolic extract showed good, while acetonic and hot water extracts showed moderate and poor activities, respectively at the concentration tested. However, at 0.125 to 1.0 mg/ml, L-ascorbic acid and kojic acid showed the excellent tyrosinase inhibitory activities of 75.12 to 92.74% and 91.23 to 99.00%.

The inhibition of tyrosinase ability might depend on the hydroxyl groups of the phenolic compounds of the mushroom extracts that could form a hydrogen bond to active site of the enzyme, leading to a lower enzymatic activity. Some tyrosinase inhibitors act through hydroxyl groups that bind to the active site on tyrosinase, resulting in steric hindrance or changed conformation (Baek et al., 2008). Gallic acid proved to be effective inhibitors of tyrosinase activity, as reported by many authors (Kubo et al., 2003; Momtaz et al., 2008). The antioxidant activity may also be one of the important mechanisms for tyrosinase inhibitory activity.

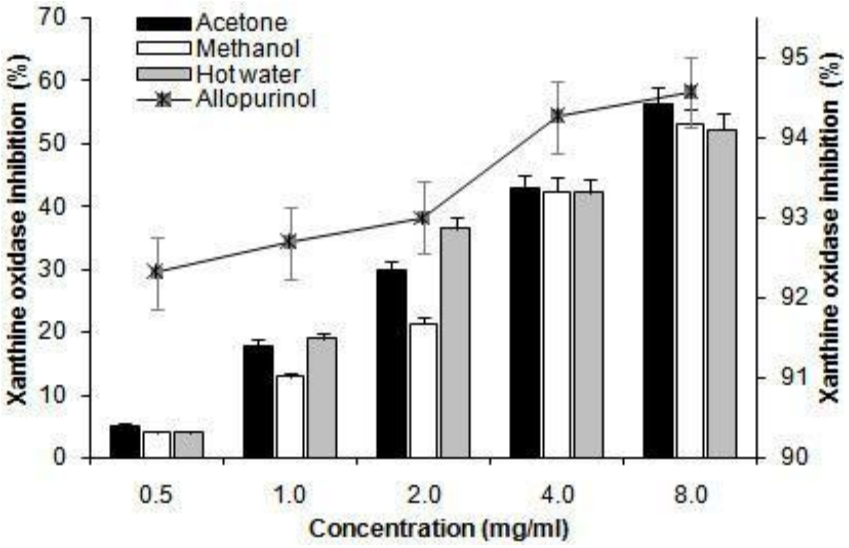

Figure 4. Xanthine oxidase inhibition activity of various extracts from the fruiting bodies of *Pleurotus salmoneostramineus*. Values expressed as means ± SE (n = 3).

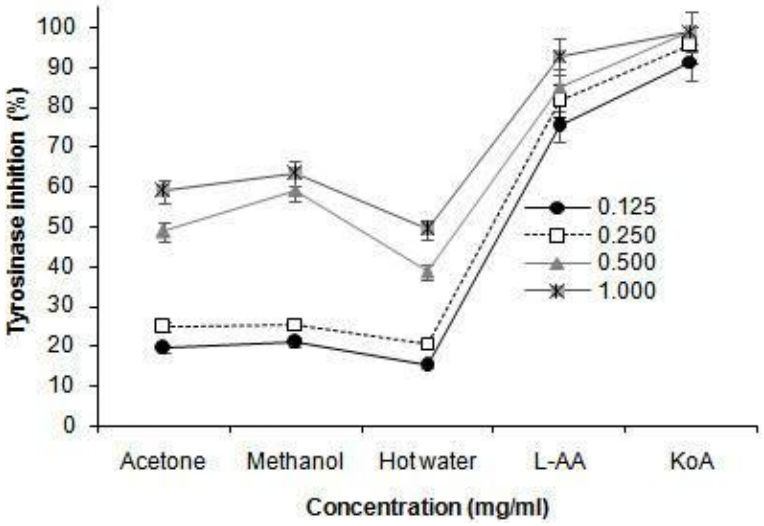

Figure 5. Tyrosinase inhibition activity of various extracts from the fruiting bodies of *Pleurotus salmoneostramineus*. Values expressed as means ± SE (n = 3). L-AA, L-ascorbic acid; KoA, kojic acid.

Conclusion

P. salmoneostramineus is an edible and medicinal mushroom, relatively new and commercially available in Korea. The study showed that *P. salmoneostramineus* had higher chelating effects on ferrous ions compared with that of synthetic antioxidant, BHT and TOC. The high phenolic content exhibited good antioxidant and antityrosinase activities. On the basis of the results, it is suggested that *P. salmoneostramineus* might be beneficial to the antioxidant, xanthine oxidase, and tyrosinase protection system of the human body against oxidative damage and others complications. This study is the first report on antioxidant and antityrosinase activities and the detection of phenolic compounds of *P. salmoneostramineus*.

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REFERENCES

Baek HS, Rho HS, Yoo JW, Ahn SM, Lee JY, Lee J, Kim MK, Kim DH, Chang IS (2008). The inhibitory effect of new hydroxamic acid derivatives on melanogenesis. Bull. Korean Chem. Soc., 29: 43-46.

Bao D, Kinugasa S, Kitamoto Y (2004). The biological species of oyster mushrooms (*Pleurotus spp.*) from Asia based on mating compatibility tests. J. Wood Sci., 50: 162-168.

Barros L, Ferreira MJ, Queiros B, Ferreira IC, Bapista P (2007). Total phenols, ascorbic acid, -carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chem., 103: 413-419.

Chang WS, Lee YJ, Lu FJ, Chaing HC (1993). Inhibitory effects of flavonoids on xanthine oxidase. Anti. Res., 13: 2165-2170.

Costantino L, Albasini A, Rastelli G, Benvenuti S (1992). Activity of polyphenolic crude extracts as scavengers of superoxide radicals and inhibitors of xanthine oxidase. Planta Med., 58: 342-344.

Cuendet M, Hostettmann K, Potterat O, Dyatmiko W (1997). Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. Helvetica Chimi. Acta, 80: 1144-1152.

Dapkevicius A, Venskutonis R, van Beek TA, Linssen JP (1998). Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. J. Sci. Food Agric., 77: 140-146.

Dinis TC, Madeira VM, Almeida LM (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-amino salicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys., 315: 161-169.

Duan X, Wu G, Jiang Y (2007). Evaluation of antioxidant properties of lichi fruit phenolics in relation to pericarp browning prevention. Molecules, 12: 759-771.

Frankel EN (1991). Recent advances in lipid oxidation. J. Sci. Food Agric., 54: 495-511.

Gulcin I, Buyukokuroglu ME, Oktay M, Kufrevioglu OI (2003). Antioxidant and analgesic activities of turpentine of *Pinus nigra* Arn. subsp. *pallsiana* (Lamb.) Holmboe. J. Ethnopharmacol., 86: 51-58.

Herraiz T, Galisteo J, Chamorro C (2003). L-tryptophan reacts with naturally occurring and food-occurring phenolic aldehydes to give phenolic tetrahydro- -caroline alkaloids: activity as antioxidants and free radical scavengers. J. Agric. Food Chem., 51: 2168-2173.

Jainu M, Shyamala DCS (2005). *In vitro* and *in vivo* evaluation of free radical scavenging potential of *Cissus quadrangularis*. Afr. J. Biomed. Res., 8: 95-99.

Kim EH, Kim SH, Chung JI, Chi HY, Kim JA, Chung IM (2006). Analysis phenolic compounds and isoflavones in soybean seeds (*Glycine max* (L.) Merill) and sprouts grown under different conditions. Eur. Food Res. Technol., 222: 201-208.

Kim MY, Seguin P, Ahn JK, Kim JJ, Chun SC, Kim EH, Seo SH, Kang EY, Kim SL, Park YJ, Ro HM, Chung IM (2008). Phenolic compound concentration and antioxidant activities of edible and medicinal mushrooms from Korea. J. Agric. Food Chem., 56: 7265-7270.

Kubo I, Chen QX, Nihei KI (2003). Molecular design of antibrowning agents: antioxidative tyrosinase inhibitors. Food Chem., 81: 241-247.

Lee YL, Huang GW, Liang ZC, Mau JL (2007). Antioxidant properties of three extracts from *Pleurotus citrinopileatus*. LWT-Food Sci. Technol., 40: 823-833.

Lio M, Moriyama A, Matsumoto Y, Takaki N, Fukumoto M (1985). Inhibition of xanthine oxidase by flavonoids. J. Agric. Biol. Chem., 49: 2173-2176.

Masuda T, Yamashita D, Takeda Y, Yonemori S (2005). Screening for tyrosinase inhibitors among extracts of seashore plants and identification of potent inhibitors from *Garcinia subelliptica*. Biosci. Biotechnol. Biochem., 69: 197-201.

Mau JL, Tsai SY, Tseng YH, Huang SJ (2005). Antioxidant properties of hot water extracts from *Ganoderma tsugae* Murrill. LWT-Food Sci. Technol., 38: 589-597.

Momtaz S, Mapunya BM, Houghton PJ, Edgerly C, Hussein A, Naidoo S, Lall N (2008). Tyrosinase inhibition by extracts and constituents of *Sideroxylon inerme* L. stem bark, used in South Africa for skin lightening. J. Ethnopharmacol., 119: 507-512.

Murakami S, Kakemaru T (1990). Genetic studies of *P. salmoneostramineus* forming albino basidiocarp. Rept. Tottori Mycol. Inst., 28: 199-204.

Owen PL, Johns T (1999). Xanthine oxidase inhibitory activity of North-Eastern North American plant remedies used for gout. J. Ethnopharmacol., 64: 149-160.

Pan Y, Wang K, Huang S, Wang H, Mu X, He C, Ji X, Zhang J, Huang F (2008). Antioxidant activity of microwave-assisted extract of longan (*Dimocarpus longan* Lour.) peel. Food Chem., 106:1264-1270.

Prasad KN, Divakar S, Shivamurthy GR, Aradhya SM (2005). Isolation of a free radical scavenging antioxidant from water spinach (*Ipomoea aquatica* Forsk). J. Sci. Food Agric., 85: 1461-1468.

Rackova L, Oblozinsky M, Kostalova D, Kettmann V, Bezakova L (2007). Free radical scavenging activity and lipoxygenase inhibition of *Mahonia aquifolium* extract and isoquinoline alkaloids. J. Inflam., 4: 1-7.

Rangkadilok N, Sitthimonchai S, Worasuttayangkurn L, Mahidol C, Ruchirawat M, Satayavivad J (2007). Evaluation of free radical scavenging and antityrosinase activities of standardized longan fruit extract. Food Chem. Toxicol., 45: 328-336.

Shahidi F, Wanasundara PK (1992). Phenolic antioxidants. Crit. Rev. Food Sci. Nutr., 32: 67-103.

Shibata N, Gohow M, Inoue T, Nagano C, Inaba K, Takekuma H, Takekuma SI, Yoshida ZI, Kai Y (1997). Crystallization and preliminary crystallographic studies of pink color chromoprotein from *Pleurotus salmoneostramineus* L. Vass. Acta Cryst., 53: 335-336.

Shimada K, Fujikawa K, Yahara K, Nakamura T (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem., 40: 945-948.

Tsai SY, Huang SJ, Mau JL (2006). Antioxidant properties of hot water extracts from *Agrocybe cylindracea*. Food Chem., 98: 670-677.

Wiseman H, Halliwell B (1996). Damage to DNA by reactive oxygen and nitrogen species: Role of inflammatory disease and progression to cancer. Biochem. J., 313: 17-29.

Yamaguchi R, Tatsumi Y, Asano M, Kato K, Ueno Y (1988). Effect of metal salts and fructose on the autoxidation of methyl linoleate in emulsions. Agric. Biol. Chem., 52: 849-850.

Zhao M, Yang B, Wang J, Li B, Jiang Y (2006). Identification of the major flavonoids from pericarp tissues of lychee fruit in relation to their antioxidant activities. Food Chem., 98: 539-544.